ATTORNEY'S DOCKET NO: **010969**

U.S. DEPARTMENT OF COMMERCE, PATENT AND TRADEMARK OFFICE		DATE: **August 13, 2001**
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLN. NO. (if known): **09/926005**
INTERNATIONAL APPLICATION NO.: **PCT/JP99/00648**	INTERNATIONAL FILING DATE: **FEBRUARY 15, 1999**	PRIORITY DATE CLAIMED:

TITLE OF INVENTION: ***ADP-RIBOSYLATION INHIBITOR COMPRISING PROANTHOCYANIDIN AS AN ACTIVE INGREDIENT AND A COMPOSITION FOR TREATING ENTEROTOXIN TYPE BACTERIAL INFECTIOUS DISEASE***

APPLICANT(S) FOR DO/EO/US: **Masatoshi NODA, Tomomasa KANDA, Akio YANAGIDA and Kazuo HIEDA**

Applicant hereby submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. XX This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ___ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. XX This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

4. XX A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. XX A copy of the International Application as filed (35 U.S.C. 371(c)(2)):

   a. ___ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. XX has been transmitted by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. XX A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. XX Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))

   a. ___ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ___ have been transmitted by the International Bureau.
   c. ___ have not been made; however, the time limit for making such amendments has NOT expired.
   d. XX have not been made and will not be made.

8. ___ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. _ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ___ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

ITEMS 11. TO 16. BELOW CONCERN OTHER DOCUMENT(S) OR INFORMATION INCLUDED:

11. XX An Information Disclosure Statement under 37 CFR 1.97 and 1.98 together with the international search report and 4 references.

12. _ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
ASSIGNEES NAMES AND ADDRESSES: ***(1) THE NIKKA WHISKY DISTILLING CO., LTD., Tokyo, Japan; and (2) MASATOSHI NODA, Chiba, Japan***
Please publish the assignee data with the application.

13. XX A **FIRST** preliminary amendment.
___ A **SECOND** or **SUBSEQUENT** preliminary amendment

14. ___ A substitute specification.

15. ___ A change of power of attorney and/or address letter.

16. XX Other items or information: 1 sheet of drawings.

U.S. APPLICATION NO. (if known) 09/926005	INTERNATIONAL APPLICATION NO. **PCT/JP99/00648**	DATE: **August 13, 2001**

17. X The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO: $860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1000.00		
International preliminary examination fee (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than __ 20 X 30 months from the earliest claimed priority date (37 DVR 1.492(e)).	**$ 130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
TOTAL	11 -20 =		X $ 18.00		
INDEPENDENT	3 - 3 =		X $ 80.00		
Multiple dependent claims(s) (if applicable)			+ $270.00		
TOTAL OF ABOVE CALCULATIONS =				**$ 990.00**	
Reduction by 1/2 for filing by small entity, if applicable. (Note 37 CFR 1.9, 1.27, 1.28).					
SUBTOTAL =				**$ 990.00**	
Processing fee of **$130.00** for furnishing the English translation later than __ 20 __ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					
TOTAL NATIONAL FEE =				**$ 990.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 per property +**					
TOTAL FEES ENCLOSED =				**$ 990.00**	
				Amount to be: refunded	$
				charged	$

ATTORNEY'S DOCKET NO: **010969**

U.S. APPLICATION NO. (if known)	INTERNATIONAL APPLICATION NO. **PCT/JP99/00648**	D ATE: **August 13, 2001**

a. XX A check in the amount of $990.00 to cover the above fees is enclosed. ($860.00 for basic filing fee and $130.00 for late filing of the declaration). (This paper is filed in triplicate)

b. ___ Please charge my Deposit Account No. 01-2340 in the amount of $___ to cover the above fees. (A duplicate copy of this sheet is enclosed.)

c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2340.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed to request that the application be restored to pending status.

Send All Correspondence To:

23850

PATENT TRADEMARK OFFICE

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **Masatoshi NODA et al.**

Serial Number: **Not Yet Assigned**
(PCT/JP99/00648)

Filed: **August 13, 2001**

For: **ADP-RIBOSYLATION INHIBITOR COMPRISING PROANTHOCYANIDIN AS AN ACTIVE INGREDIENT AND A COMPOSITION FOR TREATING ENTEROTOXIN TYPE BACTERIAL INFECTIOUS DISEASE**

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

August 13, 2001

Sir:

Prior to the calculation of the filing fees of the above application, please amend the application as follows:

IN THE CLAIMS:

Please amend the claims 4-6 and 9-11 as follows:

4. (Amended) An ADP-ribosylation inhibitor according to claim 1, wherein proanthocyanidin is the one extracted with at least one solvent selected from water, an alcohol, an ester and a ketone.

5. (Amended) An ADP-ribosylation inhibitor according to claim 1, wherein proanthocyanidin is the one purified using a styrene type adsorption resin, an anionic exchange resin,

an octadecyl-chemically binding type silica gel, an octyl-chemically binding type silica gel, a phenyl-chemically binding type silica gel and a silica gel.

6. (Amended) A composition for the treatment and/or prevention of diphtheria, pertussis, tetanus and opportunistic infection, comprising as an effective ingredient an ADP-ribosylation inhibitor comprising proanthocyanidin as an effective ingredient.

9. (Amended) A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to claim 7, wherein said edible plant or edible plant-derived material is an extract from an apple or a grape.

10. (Amended) A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to claim 7, wherein proanthocyanidin is the one extracted with at least one solvent selected from water, an alcohol, an ester and a ketone.

11. (Amended) A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to claim 7, wherein proanthocyanidin is the one purified using a styrene type adsorption resin, an anionic exchange resin, an octadecyl-chemically binding type silica gel, an octyl-chemically binding type silica gel, a phenyl-chemical type binding silica gel and a silica gel.

REMARKS

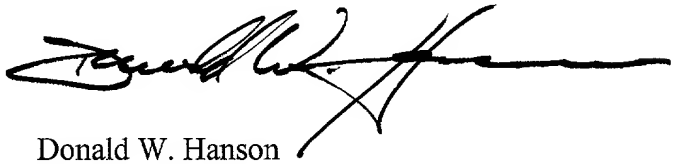
The above amendment is believed to place the claims in proper condition for examination. Early and favorable action is awaited.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

In the event there are any additional fees required, please charge our Deposit Account No. 01-2340.

Respectfully submitted,

ARMSTRONG, WESTERMAN, HATTORI,
McLELAND & NAUGHTON, LLP



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 4-6 and 9-11 have been amended as follows:

4. (Amended) An ADP-ribosylation inhibitor according to ~~any one of claims 1 through 3~~ claim 1, wherein proanthocyanidin is the one extracted with at least one solvent selected from water, an alcohol, an ester and a ketone.

5. (Amended) An ADP-ribosylation inhibitor according to ~~any one of claims 1 through 4~~ claim 1, wherein proanthocyanidin is the one purified using a styrene type adsorption resin, an anionic exchange resin, an octadecyl-chemically binding type silica gel, an octyl-chemically binding type silica gel, a phenyl-chemically binding type silica gel and a silica gel.

6. (Amended) A composition for the treatment and/or prevention of diphtheria, pertussis, tetanus and opportunistic infection, comprising as an effective ingredient an ADP-ribosylation inhibitor ~~according to any one of claims 1 through 5~~ comprising proanthocyanidin as an effective ingredient.

9. (Amended) A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to claim 7 ~~or 8~~, wherein said edible plant or edible plant-derived material is an extract from an apple or a grape.

10. (Amended) A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to ~~any one of claims 7 through 9~~ claim 7, wherein proanthocyanidin is the one extracted with at least one solvent selected from water, an alcohol, an ester and a ketone.

11. (Amended) A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to ~~any one of claims 7 through 10~~ claim 7, wherein proanthocyanidin is the one purified using a styrene type adsorption resin, an anionic exchange resin, an octadecyl-chemically binding type silica gel, an octyl-chemically binding type silica gel, a phenyl-chemical type binding silica gel and a silica gel.

DESCRIPTION

ADP-RIBOSYLATION INHIBITOR COMPRISING PROANTHOCYANIDIN AS AN ACTIVE INGREDIENT AND A COMPOSITION FOR TREATING ENTEROTOXIN TYPE BACTERIAL INFECTIOUS DISEASE

FIELD OF THE INVENTION

The present invention relates to an adenosine-5'-diphosphate (ADP)-ribosylation inhibitor comprising proanthocyanidin as an active ingredient. More particularly, the invention relates to a composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease as well as use of proanthocyanidin as a food additive and/or as a foodstuff.

BACKGROUND OF THE INVENTION

The 1998 WHO data indicate that the death toll from bacterial infections reached 20 millions all over the world. In particular, the report says that the victims of cholera amount to several million individuals per year worldwide. Even now when medical technologies involving antibiotics, vaccines or the like have been greatly improved, the number of patients has not been reduced. Furthermore, because of increased personal exchange and physical distribution accompanied by remarkable progress of international transportation in these years, traveler's diarrhea caused by Vibrio cholerae, a dysentery bacillus, toxigenic Escherichia coli, salmonella, etc. tend to be increasing even in developed countries.

Cholera is an infectious disease with a very high rate of fatality, which is caused by Vibrio cholerae and characterized by copious watery diarrhea as a major syndrome. Cholera is found all over the world, especially in Asia and Africa. Cholera is caused by such a mechanism that Vibrio cholerae enters the body via the mouth, causes an infection in the mucous membranes lining the lumen of the small intestine and proliferates to produce cholera toxin. Cholera toxin has a structure composed of two protein subunits which are known as A and B subunits and are as seen with toxins from a dysentery bacillus, toxigenic E. coli, intestinal hemorrhagic E. coli, etc. The B subunit binds to ganglioside GM1 receptor sites on the epithelial cell of the gut lumen. It is known that the A subunit of cholera toxin having an ADP-ribosylase activity catalyzes the ADP-ribosylation that results in activation of the stimulatory GTP-binding protein of the adenylyl cyclase system, known as GS α, an increased cAMP level in the epithelial cell of the gut loop and a massive secretion of water and electrolytes, leading to watery diarrhea (Med. Immunol., 21, 359-365 (1994); Masatoshi Noda, SHONI-NAIKA (Children Internal Medicine), 28 (9), 1186-1193 (1996)).

Cholera is currently treated by transfusion which is a symptomatic therapy. Under the actual situation, there is neither method nor medicament for the basic treatment of cholera has been found. As a result of extensive studies, Masatoshi Noda who is one of the present inventors already developed a medicament that inhibits toxins produced by bacteria such as Vibrio cholerae, etc. which cause enteric infections and makes the toxins harmless, see "MEDICAMENT FOR TREATING ENTEROTOXIN TYPE BACTERIAL INFECTIONS" (Japanese Patent Application No. 6-

505177, Masatoshi Noda, Hajime Nishiya and Morimichi Yamaguchi) and "GALLIC ACID ESTER DERIVATIVES AND DRUGS CONTAINING THE SAME AS ACTIVE INGREDIENTS" (Japanese Patent Application Laid-Open No. 10-273495, Masamichi Ueno, Izumi Takai, Hiroshi Ohi, Masakazu Adachi and Masatoshi Noda). However, the former involves problems of production costs, etc. due to various limitations since herb medicines are used as major ingredients. The latter requires further investigations such as safety tests since the effective components are synthetic compounds. Thus, it will take at least several years until the latter is put into practical use. It is the state of the art that any medicament with high safety to human and derived from naturally occurring substances has not yet been provided at a low drug price for the treatment of enterotoxin type bacterial infectious disease. Therefore, it is strongly desired to provide a medicament that meets the foregoing requirements.

DISCLOSURE OF THE INVENTION

An object of the invention is to provide an ADP-ribosylation inhibitor derived from a naturally occurring substance and/or a highly safe composition for the fundamental treatment and/or prevention of enterotoxin type bacterial infectious disease, which can inhibit the activity of toxins produced by enteric infection-causing bacteria via enterotoxin type bacteria such as pathogenic vibrios (Vibrio cholerae, Vibrio parahaemolyticus) and make these toxins harmless.

The present inventors made expansive investigations on naturally occurring substances, especially those derived from edible plants, that

could inhibit ADP-ribosylation catalyzed in vivo by toxins such as cholera toxin and could suppress watery diarrhea but did not adversely affect human. As a result, a substance showing an extremely effective ADP-ribosylation inhibiting action and activity for treating enterotoxin type bacterial infectious disease has been found in the extracts of edible plants such as an apple extract, a grape extract, etc. The present invention has thus been accomplished.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 is a graph showing the relationship between the concentration of an apple extract and its inhibitory activity in TEST EXAMPLE 1.

BEST MODE FOR CARRYING OUT THE INVENTION

The apple extract which is one of the effective ingredients usable in the present invention consists essentially of polyphenols. Extensive studies on these polyphenols were already made by the present inventors with respect to compositional characteristics, safety and methods for preparation to confirm their functions and practical applicability, which was filed as Japanese Patent Application No. 7-285876. These polyphenols have been prepared by its applicant and marketed as food materials, food additives, cosmetic materials, etc. These commercial products have been actually applied to various foodstuffs, processed foods, cosmetics, etc. As a result of further extensive studies, the inventors have discovered in these polyphenols a substance showing an ADP-ribosylation inhibiting action effective for the treatment of enterotoxin type bacterial

infectious disease; the inventors have identified this substance as proanthocyanidin which is one of the polyphenols.

Proanthocyanidin is a substance of at least dimers formed by condensation or polymerization of flavan-3-ol or flavan-3,4-diol having a flavonoidal skeleton (Hemingway, R. W. et al., Chemistry and Significance of Condensed Tannins, 83-107). It is confirmed that, for example, an apple extract contains 2- to 15-meric proanthocyanidin (Ohnishi-Kameyama M. et al., Rapid Comm. Mass Spectrom., 11, 31-36 (1997)).

Any extract can be used as the effective ingredient in the present invention, so long as the extract contains 2- to 15-meric proanthocyanidin derived from plants. Any plant material can be used irrespective of kind of plant, as far as the extract can be obtained from the plant material as containing a sufficient amount of the 2- to 15-meric proanthocyanidin. These plant extracts may of course be crude extracts but are required to contain at least a definite quantity of 2- to 15-meric proanthocyanidin. The extract may also be provided for use after fractionating into 2- to 15-meric proanthocyanidin and further purifying the fractions preferably to 4- to 15-meric proanthocyanidin, more preferably to proanthocyanidin of at least hexamer (6-mer). The fractionation and extraction of these proanthocyanidins of 2- to 15-mer, preferably 4- to 15-mer, more preferably at least 6-mer can be performed according to the method described in Japanese Patent Application No. 10-184143, which is herein incorporated by reference in its entirety.

Examples of edible plants containing proanthocyanidin include, in Rosaceae, strawberries and raspberries in addition to apples. Examples of edible plants other than those in Rosaceae are grapes in Vitaceae, kiwi

fruits in Actinidia, blueberries and cranberries in Ericaceae, adzuki beans and tamarinds in Leguminosae, persimmons in Ebenaceae, bananas in Musaceae, cashew nuts in Anacardiaceae, cacaos in Sterculiaceae, Japanese oaks, evergreen oaks, chestnuts and pasanias in Fagaceae and, hops in Cannabinaceae. Among those plants, young apples removed as being superfluous, seeds contained in the residue of pressed grapes, etc. are advantageously used, in view of effective recycling of resources and a large extraction volume.

That is, the present invention provides an ADP-ribosylation inhibitor comprising as an effective ingredient proanthocyanidin derived from edible plants and a composition comprising the same for the treatment of enterotoxin type bacterial infectious disease. In addition to proanthocyanidin, the inhibitor and composition of the present invention may further contain other polyphenols, proanthocyanidin to which organic acids or sugars are bound, and solvates thereof such as hydrates. Other components obtained from the same raw material may also be contained in the inhibitor and composition of the present invention.

The pharmaceutical composition of the invention which comprises as an effective ingredient proanthocyanidin derived from edible plants is useful for the treatment and/or prevention of enterotoxin type bacterial infectious disease such as traveler's diarrhea caused by cholera or botulinus. Furthermore, since the pharmaceutical composition of the invention provides an excellent effect as an ADP-ribosylation inhibitor, the composition is also effective for the treatment and/or prevention of other infectious disease such as diphtheria, pertussis, tetanus, opportunistic infection, etc., the outbreaks of which are known to be closely associated

with ADP-ribosylase.

The pharmaceutical composition of the invention comprising as an effective ingredient proanthocyanidin derived from edible plants can be prepared using carriers, excipients and other additives conventionally used to make pharmaceutical preparations. Either solid or liquid carriers and excipients may be used for making pharmaceutical preparations. Examples of such carriers and excipients include lactose, sucrose, fatty acid esters, dextrin, magnesium stearate, starch, talc, gelatin, agar, pectin, gum arabic, olive oil, sesame oil, cacao butter, ethylene glycol, etc.

The pharmaceutical composition of the invention is orally administered generally in the form of tablets, pills, capsules, granules, powders, liquid, etc. Oral dose may be appropriately determined on a case-by-case basis, depending upon conditions, age, sex, etc. of patients but the composition of the invention may be administered to adult generally in a daily dose of 100 mg to 10 g, preferably 200 mg to 1 g, when calculated as proanthocyanidin. The composition may be administered in a bolus mode, or the daily dose is divided into several times for oral administration. Of course, the daily dose may vary depending upon various conditions and in some case it may be sufficient in a smaller dose than that given above.

For oral administration of the composition in the present invention, a solid composition is available in the form of, e.g., tablets, powders or granules. In such a solid composition, edible plant-derived proanthocyanidin is blended with at least one diluent inert to the active ingredient. Examples of the diluent include lactose, mannitol, glucose, hydroxypropyl cellulose, microcrystalline cellulose, starch, metasilicic acid and magnesium aluminate. The composition may further contain

additives other than the inert diluent, if desired and necessary. Examples of such additives include a lubricant such as magnesium stearate, a disintegrating agent such as calcium cellulose glycolate, a stabilizer such as lactose, a dissolution aid such as glutamic acid or aspartic acid. Tablets or pills may be coated, if necessary and desired, with an enteric or gastric coating film of sucrose, gelatin, hydroxypropyl cellulose, hydroxypropylmethyl cellulose phthalate, reduced palatinose, zein, etc.

A liquid composition available for oral administration containing edible plant-derived proanthocyanidin as an effective ingredient may be provided in the form of a pharmaceutically acceptable emulsion, solution, suspension, syrup, elixir, etc. These liquid preparations for oral administration may also contain an inert diluent conventionally employed, e.g., purified water or ethanol. In addition to the inert diluent above, the liquid preparations may further contain a wetting agent, a suspending agent, a wetting agent, an emulsifier, a dispersing agent, a stabilizer (e.g., lactose), an auxiliary agent such as a dissolution aid (e.g., glutamic acid, aspartic acid), a sweetener, a corrigent, flavor and a preservative.

The edible plant-derived proanthocyanidin of the invention can also be used as a food additive. In this case, prophylactic effects can be anticipated. Where the foodstuffs to be applied are liquid such as milk, beverages, juice, etc., the proanthocyanidin may be added to these liquid foodstuffs immediately before drinking. The amount of the proanthocyanidin used ranges from approximately 50 mg to 1,000 mg/100 ml of the liquid. Where the proanthocyanidin is incorporated into the staple food such as pasta, bread or rice, the proanthocyanidin can be added to the staple food in an amount of approximately 50 mg to 500mg/100 g of

the hood, when the staple is cooked or processed.

EXAMPLES

Hereinafter the present invention will be described in more detail with reference to the following EXAMPLES but is not deemed to be limited thereto.

PREPARATION EXAMPLE 1 Preparation of apple extract

After 10 kg of young apple fruits were washed, crushed and then squeezed, the resulting juice was centrifuged and filtered to give 8.5 kg of clear juice. The juice was passed through a styrene-made adsorption resin column of 1 liter volume. After washing the column with water, elution was conducted with 65% aqueous ethanol solution. The eluate thus obtained was condensed in vacuo. The condensate was spray dried to give 59.7 g of the apple extract.

PREPARATION EXAMPLE 2 Preparation of grape extract

After removing stalks, grapes were pressed and the residue of the pressed grapes was sieved to collect seeds. After 1 kg of the seeds were washed with water, the seeds were extracted with 50% ethanol. The extract was condensed in vacuo. The condensate was filtered to give the extract. The extract was then passed through a styrene-made adsorption resin column of 1 liter volume. After washing the column with water, elution was conducted with 65% aqueous ethanol solution. The eluate thus obtained was concentrated in vacuo and then spray dried to give 40.7 g of the grape extract.

PREPARATION EXAMPLE 3 Fractionation of the apple extract

An aqueous solution of the apple extract obtained in PREPARATION EXAMPLE 1 was passed through a styrene-made adsorption resin column to give the total proanthocyanidin fraction containing 89% proanthocyanidin. The total proanthocyanidin fraction was applied to high performance liquid chromatography to fractionate into the monomer (catechins), dimer, trimer, tetramer and pentamer fractions and the fraction of hexamer or larger, respectively. Each of these fractions was concentrated in vacuo and lyophilized to give the dry product, respectively.

PHARMACEUTICAL PREPARATION 1

The apple extract, 50 g, obtained in PREPARATION EXAMPLE 1 was mixed with 30 g of corn starch, 10 g of crystalline cellulose, 4 g of calcium carboxymethyl cellulose and 1 g of magnesium stearate. The mixture was tableted with a tableting machine to give tablets.

PHARMACEUTICAL PREPARATION 2

The apple extract, 50 g, obtained in PREPARATION EXAMPLE 1 was mixed with 7 g of calcium carboxymethyl cellulose, 2 g of magnesium stearate and 1 g of silicic anhydride. A sufficient quantity of sterile water was added to the mixture. The mixture was extruded for granulation and dried to give granules.

PHARMACEUTICAL PREPARATION 3

The apple extract, 45 g, obtained in PREPARATION EXAMPLE 1 was mixed with 5 g of sucrose fatty acid ester. The mixture was tableted with a tableting machine to give tablets. The tablets were coated with 25 g of reduced palatinose and further with 2 g of zein to give dragees.

TEST EXAMPLE 1 Assessment by the agmatine assay

It is known that cholera toxin ADP-ribosylates Gs α,which is intracellular protein G, and also has a ADP-ribosyltransferase activity from NAD to ADP-ribose as a substrate for agmatine. Based on this mechanism, the activity of proanthocyanidin for inhibiting cholera toxin was assessed according to the agmatine assay reported by Noda et al. (Noda M. et al., Biochemistry, 28, 7936 (1989)).

That is, 1 μ g of cholera toxin and a given amount of sample shown in Table 1 were mixed with the reaction mixture of 50 mM potassium phosphate buffer (pH 7.5), 5 mM $MgCl_2$, 100 μM GTP, 100 μM [adenine-^{14}C] NAD (6000 cpm), 20 mM DTT, 20 mM agmatine and 0.1 mg/ml of ovalbumin (300 μl in total). The mixture was reacted at 30℃ for 3 hours. The reaction mixture, 50 μl, was passed through a column (ϕ5 x 20 mm) packed with Dowex AG1-X2 (manufactured by Bio Rad, Inc.) . After unreacted [adenine-^{14}C] NAD was removed, the radioactivity of [adenine-^{14}C] ADP-ribosylagmatine produced by ADP-ribosylation of cholera toxin was assayed. Using as an index this [adenine-^{14}C] ADP-ribosylagmatine formation, the inhibition of the ADP-ribosyltransferase activity of cholera toxin by each sample was determined. The results are shown in Table 1. A graph showing the relationship between the concentration of the apple extract and the ADP-ribosylation inhibiting activity is also given in Fig. 1.

Table 1

Cholera toxin inhibition rate (%)

Sample tested	Concentration (μg/ml)	Inhibition rate (%)
Apple extract (young fruit)*1	25	95.4
Grape extract (seeds)	63	96.1
Sunflower extract (seeds)	167	4.0
Rhubarb extract (herb medicine)*2	10	99.3
Total proanthocyanidin fraction	25	97.8
monomer (catechins)	25	0.0
dimer fraction	25	7.0
trimer fraction	25	9.5
tetramer fraction	25	51.8
pentamer fraction	25	96.7
fraction of $\leqq$ hexamer	25	97.6

*1: apple extract IC 50 < 10 μg/ml

*2: patients with positive response to cholera toxin: Japanese Patent Application No. 6-505177

In this test, the apple extract and the grape extract contained 51% and 63% of proanthocyanidin, respectively, when calculated as proanthocyanidin B2 according to the Porter method.

TEST EXAMPLE 2 Assay by the ligated rabbit intestinal loop method

It is known that cholera toxin ADP-ribosylates Gs α known as intracellular protein G that results in persistent activation of adenylyl cyclase, an increased cAMP level in the epithelial cell of the gut loop and a massive secretion of water and electrolytes, leading to watery diarrhea. In

order to determine the activity of inhibiting diarrhea caused by cholera toxin, the fluid accumulation activity of the apple extract in watery diarrhea was examined by the ligated rabbit loop method reported by De et al. (De S.N. and D.N. Chatterje, J. Pathol. Bacteriol., 66, 559 (1953)). That is, the rabbit ileum was ligated at every 15 cm to form several loops of the closed gut system. After 1 μ g of cholera toxin and a specified dose of the sample were given to each loop, the gut loop was returned into the body of the rabbit. The rabbit was kept as comfortable as possible and the fluid accumulation caused by cholera toxin was examined 18 hours later.

The results reveal that the apple extract suppressed almost completely the fluid accumulation caused by 1 μ g of cholera toxin at a dose of 50 μ g/loop. It was verified from these results that the edible plant extract described in the present invention and its effective ingredient proanthocyanidin act on cholera toxin to inhibit the ADP-ribosyltransferase activity and thus exhibit anti-cholera toxin activity, as does the extract of herb medicine "rhubarb".

INDUSTRIAL APPLICABILITY

The ADP-ribosylation inhibitor of the invention comprising proanthocyanidin as an active ingredient is effective for the treatment and/or prevention of enterotoxin type bacterial infectious disease. The safety of proanthocyanidin as the active ingredient which is contained in such edible plant extracts as described above has already been established, since plants from which proanthocyanidin is derived have been utilized as edible plants in the long history of man. Even so, the safety of proanthocyanidin has been confirmed again prior to its use as a food

additive by acute toxicity tests, etc. Proanthocyanidin has already been used widely as an extract from edible plants, an antioxidant or a food material. Any adverse effect to human is not reported at all. Thus, proanthocyanidin is extremely safe, very effective for the treatment and/or prevention of enterotoxin type bacterial infectious disease. Therefore, proanthocyanidin can provide an extremely useful medicament.

CLAIMS

1. An adenosine-5'-diphosphate (ADP)-ribosylation inhibitor comprising proanthocyanidin as an effective ingredient.

2. An ADP-ribosylation inhibitor according to claim 1, wherein proanthocyanidin is obtained from an edible plant or an edible plant-derived material.

3. An ADP-ribosylation inhibitor according to claim 2, wherein said edible plant or edible plant-derived material is an extract from an apple or a grape.

4. An ADP-ribosylation inhibitor according to any one of claims 1 through 3, wherein proanthocyanidin is the one extracted with at least one solvent selected from water, an alcohol, an ester and a ketone.

5. An ADP-ribosylation inhibitor according to any one of claims 1 through 4, wherein proanthocyanidin is the one purified using a styrene type adsorption resin, an anionic exchange resin, an octadecyl-chemically binding type silica gel, an octyl-chemically binding type silica gel, a phenyl-chemically binding type silica gel and a silica gel.

6. A composition for the treatment and/or prevention of diphtheria, pertussis, tetanus and opportunistic infection, comprising as an effective ingredient an ADP-ribosylation inhibitor according to any one of claims 1 through 5.

7. A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease comprising proanthocyanidin as an effective ingredient.

8. A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to claim 7, which is

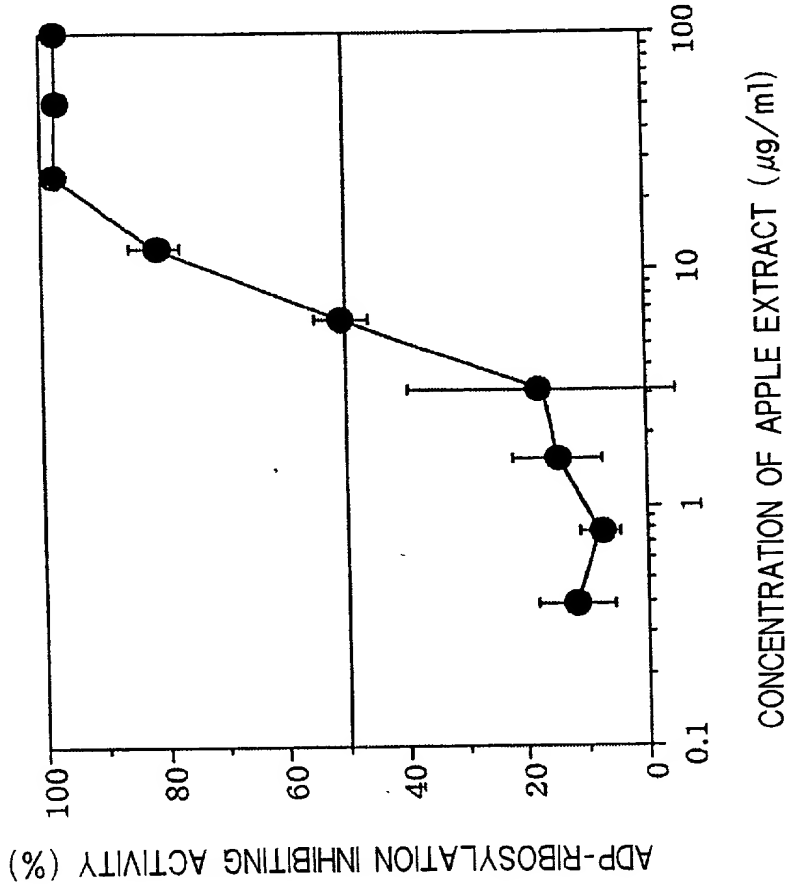
for the treatment and/or prevention of cholera, botulinus and traveler's diarrhea.

9. A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to claim 7 or 8, wherein said edible plant or edible plant-derived material is an extract from an apple or a grape.

10. A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to any one of claims 7 through 9, wherein proanthocyanidin is the one extracted with at least one solvent selected from water, an alcohol, an ester and a ketone.

11. A composition for the treatment and/or prevention of enterotoxin type bacterial infectious disease according to any one of claims 7 through 10, wherein proanthocyanidin is the one purified using a styrene type adsorption resin, an anionic exchange resin, an octadecyl-chemically binding type silica gel, an octyl-chemically binding type silica gel, a phenyl-chemical type binding silica gel and a silica gel.

FIG.1


ADP-RIBOSYLATION INHIBITING ACTIVITY (%)
100
80
60
40
20
0
0.1
1
10
100
CONCENTRATION OF APPLE EXTRACT (μg/ml)

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Declaration and Power of Attorney for U.S. Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者であると（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor(if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

ADP-RIBOSYLATION INHIBITOR COMPRISING PROANTHOCYANIDIN AS AN ACTIVE INGREDIENT AND A COMPOSITION FOR TREATING ENTEROTOXIN TYPE BACTERIAL INFECTIOUS DISEASE

上記発明の明細書（下記の欄で×印がついいないい場合は、本書に添付）は、

the specification of which is attached hereto unless the following box is checked:

☐ ___月___日に提出され、米国出願または特許協定条約国際出願番号を_____ とし、（該当する場合）_____ に訂正されました。

☐ was filed on Feb. 15, 1999 as United States Application Number or PCT international Application Number PCT/JP99/00648 and was amended on _____ (if applicable).

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第３７編第１条５６項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

Japanese Language Declaration
(日本語宣言書)

私は、米国法典第３５編１１９条(a)-(d)項又は３６５条(b)項に基づき下記の、米国以外の少なくとも一カ国を指定している特許協力条約３６５(a)項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

I hereby claim foreign priority under Title 35, United States Code, Section 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s) 外国での先行出願			Priority Not Claimed 優先権主張なし
(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	☐
(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	☐

私は、第３５編米国法典１１９条(e)項に基づいて下記の米国特許出願規定に記載された権利をここに主張いたします。

I hereby claim the benefit under Title 35, United States Code, Section 119(e)of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Application No.) (出願番号)	(Filing Date) (出願日)

私は、下記の米国法典第３５編１２０条に基づいて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約３６５条(c)に基づく権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第３５編１１２条第１項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第３７編１条５６項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code Section 112. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filling date of the prior application and the national or PCT international filing date of application.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)
(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)

私は、私自身の知識に基づいて本宣言書中で私が行う表明が真実であり、かつ私の入手した情報と私の信じるところに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第１８編第１００１条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行えば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Japanese Language Declaration
（日本語宣言書）

委任状： 私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁護士または代理人として、下記の者を指名いたします。（弁護士、または代理人の氏名及び登録番号を明記のこと）

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith (list name and registration number)
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